Azole Resistance in *Candida albicans* from an HIV+ Patient: Upregulation of Lanosterol Demethylase mRNA

T. C. White[1], J. Rhine-Chalberg[2], M. A. Pfaller[2], M. Rinaldi[3] and S. Reading[3]

[1]Presenting author; Department of Pharmaceutical Chemistry, University of California at San Francisco

[2]Department of Pathology, Oregon Health Sciences University

[3]Departments of General Practise, Medicine and Pathology, University of Texas Health Science Center at San Antonio

Candida albicans is a dimorphic yeast which is present as a commensal infection in over 50% of healthy adults. Under conditions in which the immune system is compromised, *Candida* can cause oral, esophogeal, vaginal and systemic candidiasis. Oral candidiasis is the most frequent and the earliest infection detected in HIV+ patients with CD4 cell counts less than 500. After an initial bout of candidiasis, the HIV+ patient is frequently placed on prophylactic azole drugs to prevent recurrence. In addition. azole prophylaxis is used to prevent the recurrence of cryptococcal meningitis. The treatment of patients with low levels of azole drugs for long periods of time may allow for the emergence of azole resistant *Candida* strains. Recently, such strains have begun to appear.

Azole drugs, including clotrimazole, ketoconazole and fluconazole, are thought to act on lanosterol demethylase (LDM), a cytochrome P450 containing enzyme in the biosynthetic pathway leading to the synthesis of ergosterol, a major component of yeast cell membrane. The azole drugs bind to the heme of the molecule; the specificity of the drug is determined by the fit of the drug into the active site of the enzyme.

We describe one HIV+ patient who was treated for oral candidiasis with the azole drug, fluconazole. Isolates of *Candida* were taken from this patient over the course of two years (16 isolates) during which time the patient's drug regime was increased steadily. The isolated strains of *Candida albicans* show stepped increases in azole resistance from 0.25 µg/ml to >64 µg/ml.

Using a DNA fragment which has been extensively used to fingerprint strains of *Candida albicans*, we show that an unusually stable strain of *Candida albicans* was isolated at every stage. Using a DNA fragment from LDM, we show that the target gene is neither amplified nor extensively rearranged. Northern blots of total RNA from these isolates show an increase in LDM mRNA levels when the resistant levels of the isolates increase from 8.0 µg/ml to 16 µg/ml. This increase may be due to an increase in transcription or in mRNA stability. We are currently determining the levels of LDM protein in these isolates. In the near future, we will attempt to determine the DNA alteration which results in this increase in mRNA levels. We are also screening other strains of *Candida albicans* which have developed resistance to determine the mechanism(s) which result in azole drug resistance in *Candida albicans*.

Source: https://www.industrydocuments.ucsf.edu/docs/zhmv0000